WORKING DOCUMENT TO ICES WORKSHOP ON MICROINCREMENT DAILY GROWTH IN EUROPEAN ANCHOVY AND SARDINE (WKMIAS). MAZARA DEL VALLO, SICILY (ITALY), 21-25 OCTOBER 2013

Culture viability of *Sardina pilchardus*: preliminary results of growth in captivity.

José Iglesias and Lidia Fuentes

Instituto Español de Oceanografia (IEO). Centro Oceanográfico de Vigo. Subida a Radio Faro, 50. 36390. Vigo. Pontevedra. Spain.

Abstract

Larvae of *Sardina pilchardus* were obtained in captivity from fertilized eggs captured in the wild, and grown in a 10 000 L tank. *Isochrysis galbana*, *Artemia franciscana* nauplii and live zooplankton were used as prey during the first two weeks; afterwards, animals were fed on artemia metanauplius enriched with *Isochrysis galbana*. A dry feed (Gemma 0.4 and 0.8) from SKRETTING S.A. (Burgos, España) was supplied from the third to 18th month. The total length reached by sardines at one year of life was 162.02 ± 9.49 mm, corresponding to a wet weight of 36.12 ± 10.82 g. Total length at 18 months was 182.37 mm. A long experiment (18-month) of sardine culture is described for the first time, and growth data reported can contribute to determine its potential interest as a candidate for marine aquaculture.

1. Introduction

One of the most representative species in the Spanish coast regarding fishing and human consumption is sardine *Sardina pilchardus* Walbaum 1792. This is a pelagic species with a wide distribution extending in the North-East Atlantic from the Celtic Sea and North Sea in the north to Mauritania in the south. Populations of Madeira, the Azores and the Canary Islands are at the western limit of the distribution (Parrish et al. 1989). Sardine is also found in the Mediterranean and the Black seas. According to FAO (Food and Agriculture Organization), the world catch of *S. pilchardus* in 2011 was 1 036 708 tonnes.

Spain and Portugal have a major international fishing fleet dedicated to the capture of this species; the current knowledge about the identity of the sardine Iberian stock has been studied by Riveiro et al. (2012). During the last decades, sardine catches in Atlanto Iberian stock (ICES, International Council for the Exploration of the Sea, subdivisions VIIIc and IXa) presented some fluctuations, peaked in 1981 at 217 thousand tonnes, and thereafter showing a general decrease. As a result of the decline of the population, the sustainability of the fishery is considered at risk, and ICES in his last advice has recommended a drastic reduction in fishing effort (ICES 2013). The scarcity of the species has led to a huge increase in the price of sardines in 2013, which have exceeded the economic value of other species such as hake in some periods of the year (source: www.pescadegalicia.com). Consequently, this fact encouraged us to analyze the possibility of culturing this species.

Laboratory experiments using wild sardine specimens have been conducted previously: acclimation to captive conditions (Marcalo et al. 2008), induction to spawning (Olmedo et al. 1990), embryonic development (Miranda et al. 1990) and larval rearing (Blaxter 1969, Miranda et al. 1990, Miranda et al. 1992, Silva & Miranda 1992). Garrido et al. (2007) and Garrido et al. (2008) described both the feeding behaviour of sardines under culture conditions, and the diet and food intensity in the wild. In any case, none was focused on growth studies of this species at long term in captivity.

The main objective of this paper was to study the growth of sardine, *Sardina pilchardus* under culture conditions, from hatching to adult stage, in order to determine the culture viability of the species.

2. Material and methods

2.1 Eggs and larvae source

Larvae used in this experiment were from fertilized eggs collected from the wild with oblique trawls of a zooplankton net in the Moaña area (inner part of Ria de Vigo, NW Spain) on May 23th, 2010. Each trawl lasted 10 min, depth of water column was 9-15 m, water temperature 17.5°C and salinity 34.5 psu.

The zooplankton net used was 2 m in diameter with a 250 µm mesh and the final cod end of 200 µm mesh. After ruling out on board the fraction greater than 2 mm, zooplankton retained was transported in seawater to the facilities of the Spanish Institute of Oceanography (IEO) in Vigo.

2.2 Rearing conditions

Sardine eggs were placed in a 10 000 L cylindrical tank (2.60 m in diameter and 1.9 m water depth), provided with central aeration, surface inlet and a lateral surface cylindrical (60 cm high, 300 µm mesh) outlet, which was used for both incubation and larval culture. According to Rusell (1976), this species has large eggs (1.30-1.90 mm of diameter), with large perivitelline space, oil globule and segmented yolk. Once at the IEO (May 23th 2010) most of these eggs (80%) were in gastrula stage, although there were also eggs in blastocyst and embryonic stage (20%). After three days, almost all eggs had hatched; therefore, May 26th was considered as the sardine larvae hatching date in this experiment.

Daily supply of *Isochrysis galbana* was performed in order to maintain a "green water" system with an approximate concentration of 20 10^3 cells mL^{-1}. One to 6 millions of nauplius of *Artemia franciscana* Kellog, 1906, were delivered in 4 daily doses during the first weeks of larval culture. In addition, live wild zooplankton was added once a week. Thereafter, artemia metanauplius, enriched with *Isochrysis galbana* Parke, were added. From the third month onwards, only a dry feed, Gemma 0.4-0.8 (SKRETTING España S.A., Burgos, España) was supplied using surface feeders.

A daily water partial replacement was carried out during the first three months, opening the circuit with a water flow of 4 L min^{-1} during 4 hours. Afterwards, an open water system was used. Natural photoperiod was applied (16:8 L:D). Ambient temperature (13-20ºC) was used, and salinity ranged 33.5-35 psu. The oxygen, ammonium and nitrite levels were recorded daily, and pH was determined weekly.

2.3 Feeding and swimming behaviour

Observations of feeding and swimming (shoaling and distribution in the tank) behaviour of the sardines were conducted throughout the culture process.

2.4 Growth sampling

The first length sampling was performed at 23 days of age (n=5). Samplings were taken every 5 days until day 35 of life. Subsequently, samplings were conducted every 10 days (until day 55), every 15 days (up to day 160) and thereafter on a monthly basis until one year. After one year old, total length and wet weight were recorded sporadically.

Distance from back of head to first dorsal fin ray (BHFDF), distance from anus to base of caudal fin (ABCF), insertion of pelvic fin to anus (IPFA), standard length (SL) and total length (TL) (Fig. 1) were recorded individually. The first two months measurements were made under the microscope, and from day 85 onwards, a digital ictiometer and a caliper were used.

Based on recorded TL data, a growth curve and its corresponding equation were obtained.

Sampled specimens were individually preserved in absolute alcohol. An additional study of age determination and validation based on otoliths reading is being elaborated in collaboration with the ICES ageing team at the IEO.

In order not to interfere in the growing conditions of this long term experiment of 18 months, survival was not determined; only qualitative estimations based on visual observations were conducted.

3. Results and discussion

Previous studies based on stomach content have shown that wild sardines have a very diverse diet: high numbers of phytoplankton cells, mainly diatoms and dinoflagellates, usually occurs, but these generally represent <10% of total prey biovolume (Cunha et al. 2005); Garrido (2003) reported that stomach contents are volumetrically dominated by crustacean naupliar stages and small copepods, and numerically dominated by microplankton, especially chain-formed diatoms (99% during upwelling events). In accordance with this, Artemia nauplius and metanauplius, wild zooplankton, and even dry feed supplied in the present work were well accepted by the sardines; this fact is inferred by the reduction of the prey density (clearance) recorded daily. The presence of phytoplankton (*Isochrysis galbana*) in the culture tank, which is characteristic of green water system, has probably also contributed to the sardine diet. According Garrido et al. (2007) sardines use two feeding modes and switch between them depending on prey size: filter-feeding when single phytoplankton cells and zooplankton <780 µm were introduced into the tank, and particulate-feeding when bigger preys were offered.

In previous culture experiments concerning larval rearing of sardine, rotifer *Brachionus plicatilis* Müller, 1786, artemia and wild zooplankton (nauplius and juvenile copepods) had been used as diet (Miranda et al. 1992, Álvarez 2002).

Sardines behaviour at one month old was to swim erratically in small groups throughout the tank; this behaviour was modified when food was supplied only in a feeding area, and the sardines formed a unique group around it; however, this reaction was not observed when a homogeneous distribution of preys was produced by increasing the aeration intensity.

During the second and third month of life, sardines began to swim in a loose unique shoal, swimming continuously upstream (against the flow). This pattern behaviour was only modified when a disturbance occurred in the tank (sampling, food supply, etc.).

From the third month, and especially from one year old when sardines already fed on dry feed, they showed a very active feeding behaviour, similar to that of other species such as sea bass and sea bream, etc. Other authors had also used pellets to feed adult sardines captured from the sea (Garrido et al. 2007, Peleteiro et al. 2004). The sampling process is

particularly difficult during this period because of the animal elusive behaviour, which occasionally caused some individuals to jump out of the culture tank.

The highest peak in mortality was observed during the larval rearing period. Afterwards, they kept on dying at a lower rate until one year of life, when there were only 15 live individuals left. Thereafter, 1-2 specimens died every month until the end of the experiment. Throughout the ongrowing process, it was observed that certain individuals showed abnormal head with flattened nose due to collision and friction against the walls of the tank, which did not seem to affect their survival.

The growth in length (TL) from hatching to 18 months of life in captivity is shown in Fig. 2; Sardines reached a mean value of 162.02 ± 9.49 mm at one year old and 182.37 mm at 18 months old. Growth equation during this period was:

$y=52.58\text{Ln}(x)-154.6$; $R^2=0.972$

Sizes attained by wild individuals whose age was estimated by Álvarez & Alemany (1997), based on daily growth rings of the Galician sardine are lower than those obtained in cultured specimens of the present study. This higher growth in captivity agrees with previously observed for other cultured species; for example, the overall growth rates obtained by Iglesias et al. (2010) for hake maintained in captivity during a 200-day experiment were higher than those reported by Pontual et al. (2003) from tagged and released wild fish.

However, the TL value reached at 18 months in captivity (182.37 mm), is virtually the same as the average value for the year class 1 (18.2 cm) contributed by ICES (2012) for the area corresponding to the geographical position of Galicia (IXa-N).

Table 1 shows the evolution of the different measures recorded during the experiment. It is interesting to note that the back of head to first dorsal fin ray distance (BHFDF) varies substantially throughout the life cycle of the sardine (Fig. 3). From hatching until the first month of life, this distance is large and represents nearly 40% of the total length of the larva, decreasing considerably until the second month to about 15-18% of the TL; values remain constant during the phases of post-larvae, juveniles and adult stage.

Wet weights reached at 10, 12 and 18 months were 29.5±5.92, 36.12±10.82 and 37.37 g, respectively. These values are lower than those reported by ICES (2012) for the year class 1 (55 g). The reduced weight increase observed in captivity between months 12 and 18 could possibly be attributed to the commercial inert diet used in this study, which possibly was not suitable for this species, in fact it is elaborated by SKRETTING S.A. as weaning –diet for marine fish larvae with high protein requirement in general. Therefore, further studies on this subject are needed in the future.

In general, considering the growth rates in captivity, and that commercial minimum size (11 cm TL) can be achieved approximately in 6 months, sardine appears to be an interesting candidate for aquaculture. However, other aspects, such as inert diets improvement and culture process costs, must be analyzed before deciding its viability. Regardless, this culture experiment is valuable for other fields, such as age validation studies. In fact, when otoliths are analyzed, this work could have relevance for better interpreting growth data from field-collected individuals.

4. Acknowlwdgements

We appreciate the technical assistance of M. Calero and M.J. Lago during the experiences.

5. References

Álvarez F. 2002. Crecimiento diario de *Sardina pilchardus* Walbaum, 1792 (Pises: Clupeidae) y su aplicación al estudio de procesos de reclutamiento. Doctoral Thesis. University of Santiago de Compostela, Santiago de Compostela.

Álvarez F, Alemany F. 1997. Birthday analysis and its application to the study of recruitment of the Atlanto-Iberian sardine *Sardina pilchardus*. Fishery Bulletin -NOAA 95: 187-194.

Beare DJ, Burns F, Greig A, Jones EG, Peach K, Kienzle M, McKenzie E, Reid DG. 2004. Long-term increases in prevalence of North Sea fishes having southern biogeographic affinities. Marine Ecology-Progress Series 284: 269-278.

Blaxter JHS. 1969. Experimental rearing of pilchard larvae, *Sardina pilchardus*. Journal of the Marine Biological Association of the United Kingdom 49: 557-575.

Cunha ME, Garrido S, Pissarra J. 2005. The use of stomach fullness and colour indices to assess *Sardina pilchardus* feeding. Journal of the Marine Biological Association of the United Kingdom 85: 425-431.

Garrido S. 2003. Alimentação de *Sardina pilchardus* (Walbaum, 1792) ao largo da costa continental portuguesa e implicações da condição nutritional das fêmeas na qualidade dos oócitos. MS Thesis, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Porto.

Garrido S, Ben-Hamadou R, Oliveira PB, Cunha ME, Chicharo MA, van der Lingen CD. 2008. Diet and feeding intensity of sardine *Sardina pilchardus*: correlation with satellite-derived chlorophyll data. Marine Ecology-Progress Series 354: 245-256. DOI: 10.3354/meps07201.

Garrido S, Marcalo A, Zwolinski J, van der Lingen CD. 2007. Laboratory investigations on the effect of prey size and concentration on the feeding behaviour of *Sardina pilchardus*. Marine Ecology-Progress Series 330: 189-199. DOI: 10.3354/meps330189.

Gibson RN, Ezzi IA. 1990. Relative importance of prey size and concentration in determining the feeding behaviour of the herring *Clupea harengus*. Marine Biology 107: 357-362.

ICES. 2012. Report of the Working Group on Southern Horse Mackerel, Anchovy and Sardine (WGHANSA) 23 - 28 June 2012. Azores (Horta), Portugal.

ICES. 2013. Sardine in subdivisions VIIIc and IXa. Advice 2013, Book 7

Iglesias J, Lago MJ, Sánchez FJ, Cal R. 2010. Capture, transport and acclimatization to captivity of European hake, *Merluccius merluccius* L: preliminary data on feeding and growth. Aquaculture Research 41: 607-609.

Marcalo A, Pousao-Ferreira P, Mateus L, Correia JHD, Stratoudakis Y. 2008. Sardine early survival, physical condition and stress after introduction to captivity. Journal of Fish Biology 72 (1): 103-120. DOI: 10.1111/j.1095-8649.2007.01660.x.

Miranda A, Cal RM, Iglesias J. 1990. Effect of temperature on the development of eggs and larvae sardine *Sardina pilchardus* Walbaum in captivity. Journal of Experimental Marine Biology and Ecology 140: 69-77.

Miranda A, Sánchez J, Iglesias J, Alvarez F, Alemany F. 1992. Crecimiento larvario de sardina *Sardina pilchardus* (Walb.). Informes Técnicos. Instituto Español de Oceanografia 132, 15 p. Madrid. ISSN: 02121565.

Olmedo M, Iglesias J, Peleteiro JB, Forés R, Miranda A. 1990. Acclimatization and induced spawning of sardine *Sardina pilchardus* Walbaum in captivity. Journal of Experimental Marine Biology and Ecology 140: 61-67.

Parrish RH, Serra R, Grant WS. 1989. The monotypic sardines, sardine a sardinops. Their taxonomy, distribution, stock structure, and zoogeography. Canadian Journal of Fisheries and Aquatic Sciences 46(11): 2019-2036.

Peleteiro B, Marçalo A, Olmedo M, Pousão-Ferreira P, Garrido S, Porteiro C, Stratoudakis Y. 2004. Sardine tagging off the Iberian Peninsula: laboratory experiments and operations at sea. ICES ASC CM 2004/Q20.

Pontual H, Bertignac M, Battaglia A, Bavouzet G, Moguedet P, Groisson AL. 2003. A pilot tagging experiment on European hake (*Merluccius merluccius*): methodology and preliminary results. ICES Journal of Marine Science 60: 1318-1327.

Silva A, Miranda A. 1992. Laboratory rearing of sardine larvae (*Sardina pilchardus*) and early effects of starvation: a preliminary experiment. Boletín del Instituto Español de Oceanografía 8(1): 163-174.

Riveiro I, Santos MB, Silva A. 2012. Current knowledge on Iberian sardine (*Sardine pilchardus*) stock identity. Working Document to Benchmark Workshop on Pelagic Stocks, Copenhagen, February 2012.

Russell FRS. 1976. The Eggs and Planktonic Stages of British Marine Fishes. Academic Press Inc. (London) Ltd. Library of Congress Catalog Card Number 75-19670. ISBN: 0-12 604050-8. Printed in Great Britain by The Whitefriars Press Ltd. London and Tonbridge.

www.pescadegalicia.com

Figure Legends

Figure 1. Diagram of sardine postlarva to illustrate body measurements.

Figure 2. Growth in total length (TL) of *Sardina pilchardus* in captivity since 26 day until 18 month of life.

Figure 3. Variation with age of the relationship between back of head to first dorsal fin ray distance (BHFDF) and total length.

Table 1. Summary of the different measurements taken to sardine along the culture experiment.

Age (m)	TL (mm)	SL (mm)	BHFDF (mm)	ABCF (mm)	IPFA (mm)
1	23.80±4.27	19.84±5.54	7.40±1.08	3.96±1.04	6.93±1.44
3	78.17±6.40	63.83±6.52	12.20±1.30	15.83±2.56	16.00±1.41
6	130.40±2.97	107.00±4.69	21.40±0.50	26.80±1.64	28.00±0.55
9	143.4±9.91	124.60±8.35	23.20±3.30	29.40±3.13	30.60±3.08
12	162.02±9.49	141.18±9.95	26.30±2.10	35.83±3.54	38.55±2.22
18	182.37	155.44	27.97	41.41	43.10

Age (m): Age in months

TL (mm): Total length

SL (mm): Standard length

BHFDF (mm): Back of head to first dorsal fin ray

ABCF (mm): Anus to base of caudal fin

IPFA (mm): Insertion of pelvic fin to anus

Figure 1

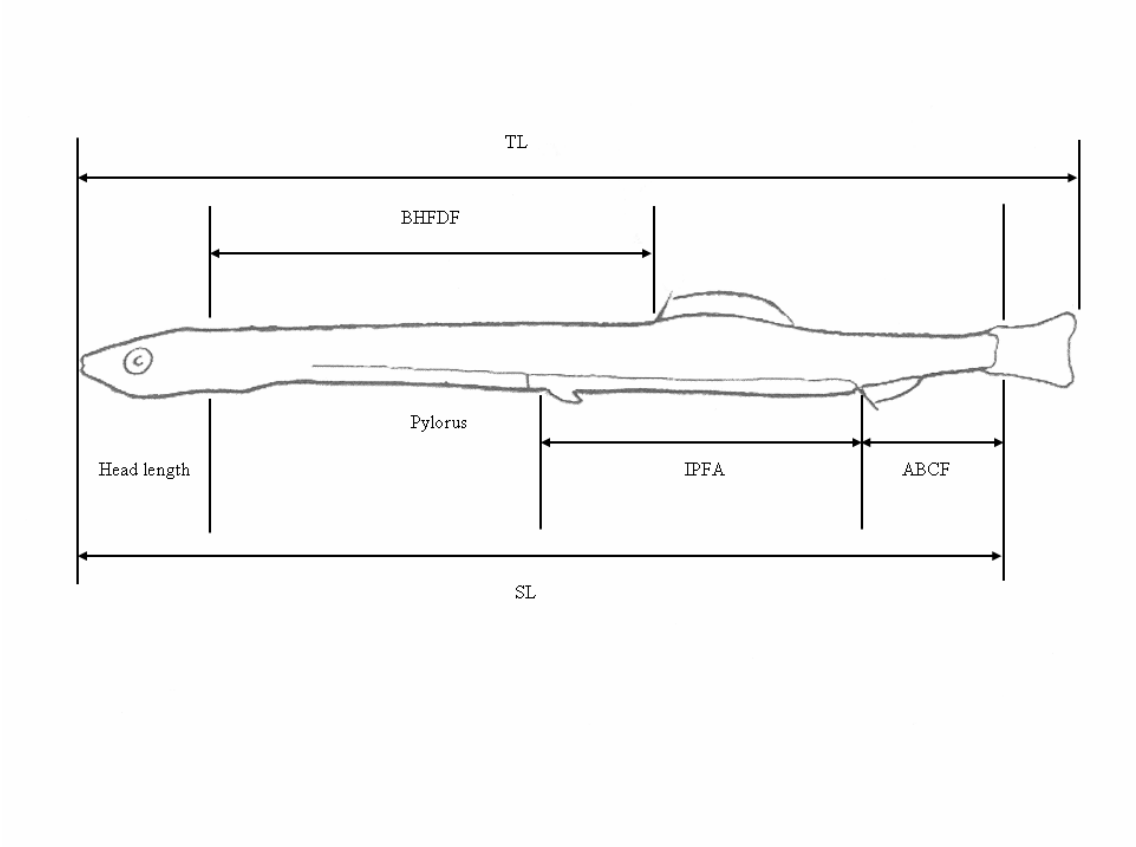


Figure 2

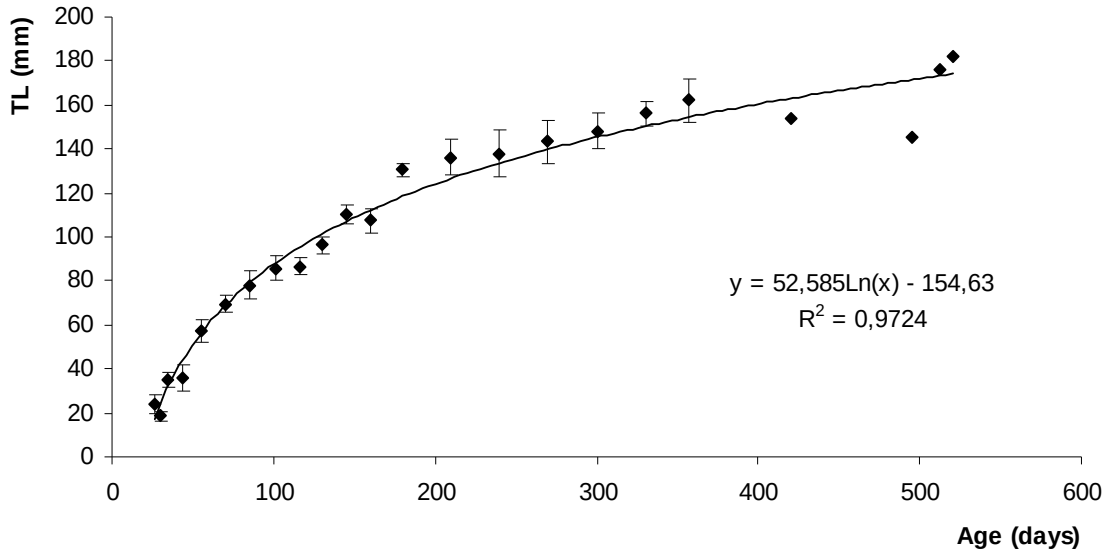
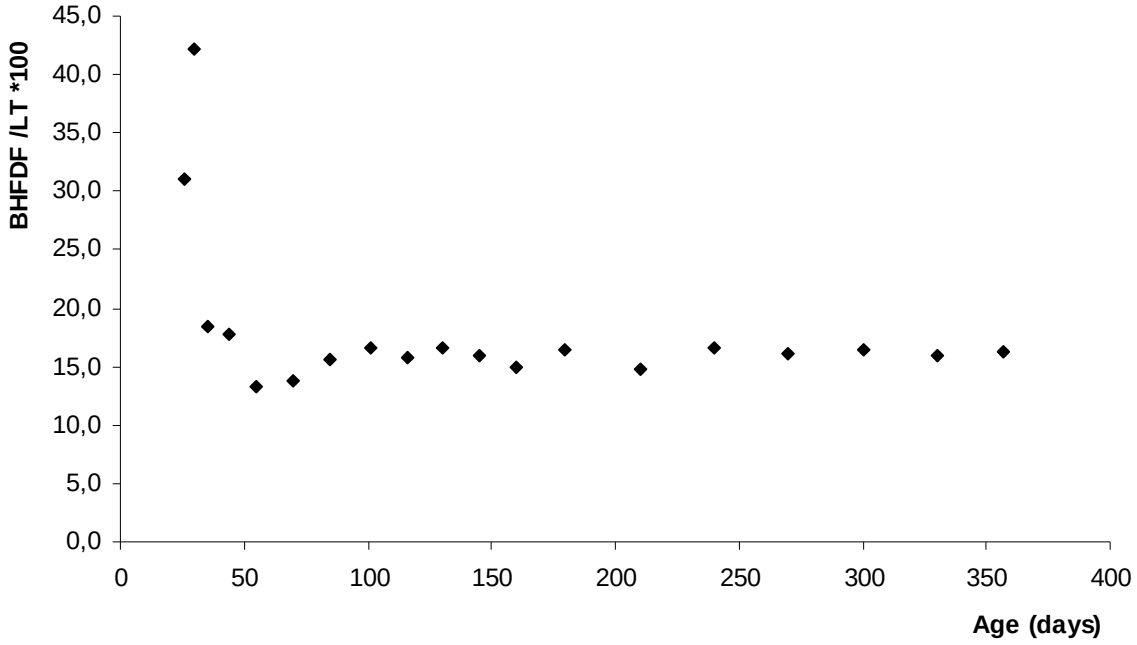

TL (mm)
200
180
160
140
120
100
80
60
40
20
0
0
100
200
300
400
500
600
Age (days)
y = 52,585Ln(x) - 154,63
R2 = 0,9724

Figure 3


45,0
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
BHFDF /LT *100
0
50
100
150
200
250
300
350
400
Age (days)